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Effects of alligator pepper (*Aframomum melegueta*) and black pepper (*Piper nigrum*) supplementation on growth performance, carcass traits, and health status of broiler chickens

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ABSTRACT

This study investigated the combined effects of alligator pepper (*Aframomum melegueta*) and black pepper (Piper nigrum) on growth performance, carcass yield, and hematological parameters of broiler chickens. A total of 160-day-old broiler chicks were assigned to four dietary treatments in a completely randomized design: a control diet and three diets containing increasing combinations of both spices. During 49-day feeding trial, the birds receiving the highest combined inclusion consistently exhibited superior growth rates, greater feed intake, and improved nutrient utilization compared to the control. Carcass evaluation revealed noticeable enhancements in dressed weight and prime cuts, suggesting increased muscle accretion. Hematological indices indicated stable values for packed cell volume, red and white blood cell counts, while neutrophil counts increased and eosinophil counts decreased in spice-supplemented groups, reflecting potential immune benefits. However, hemoglobin levels declined slightly at higher supplementation levels of alligator pepper and black pepper, although the values remained within the physiological range. Thyroid hormones levels suggested that moderate supplementation could support thyroid activity, while other endocrine parameters remained unaffected. the combined inclusion of alligator pepper and black pepper in broiler diet enhanced growth performance, carcass quality, and immune-related blood indices without compromising health, revealing its possibility as a safe and natural growth-promoting alternative in poultry production.

Keywords: Broiler chickens, Carcass traits, Growth performance, Phytogenic feed additives, Serum biochemistry

The poultry industry plays a critical task in ensuring global food security by supplying affordable, high-quality animal protein, while boosting economic growth and public health (Onogwu *et al.* 2024; Gabriel *et al.* 2025). Despite this, broiler chicken production continues to face challenges such as oxidative stress, disease outbreaks, and restrictions on antibiotic growth promoters (AGPs) due to concerns over antimicrobial resistance (Bamidele, 2019; Onogwu *et al.* 2024). These factors enforce the search for natural feed additives that can sustain performance, enhance immune function, and support livestock health without the catch of synthetics (Gabriel *et al.* 2025). Phytogenic feed additives, derived from herbs and spices, show promising alternatives to AGPs owing to their antimicrobial, antioxidant, and immunomodulatory bioactive compounds (Bamidele, 2019; Onogwu *et al.* 2024). Alligator pepper (AP) and black pepper (BP) are particularly significant: Alligator pepper is rich in essential oils, alkaloids, and flavonoids that aid digestion and enhance health (Onogwu et al. 2024), while black pepper, high in piperine, improved nutrient uptake, gut health, and antioxidant activity (Ogbuewu and Mbajiorgu, 2023). Yahaya *et al.* (2024) documented remarkably improved growth performance in broilers supplemented with black pepper. In addition, systematic reviews have revealed overall improvements in feed conversion ratios with phytogenics across commercial poultry settings (Martinez *et al.* 2022). Combining phytogenic additives may yield synergistic effects (Gabriel *et al.* 2025; Onogwu *et al.* 2024). This study examines the combined effect of alligator pepper and black pepper on growth performance, carcass traits, hematological profiles, and immune function in broilers focus to offer a natural, effective alternative to antibiotic growth promoters in poultry production.

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MATERIALS AND METHODS

Preparation of alligator pepper and black pepper: AP and BP were purchased from Ikole market, Ekiti State, Nigeria. Spices were cleaned, air-dried at ambient temperature (25–28 °C) for two weeks, and ground into fine powder using an electric grinder. The powders were stored in airtight containers to prevent moisture absorption until incorporation into experimental diets.

Experimental birds and management: A total of 160-day-old Ross 308 broiler chicks (Agrited Farm, Ibadan, Nigeria) were randomly assigned to four dietary treatments in a completely randomized design. Each treatment had four replicates of 10 birds (n = 40 per treatment). The study lasted 49 days. The treatments were:

- T1: Basal diet without AP and BP
- T2: Basal diet + 0.5 g/kg AP + 0.5 g/kg BP
- T3: Basal diet + 1.0 g/kg AP + 1.0 g/kg BP
- T4: Basal diet + 1.5 g/kg AP + 1.5 g/kg BP

All diets met NRC (1994) nutrient requirements (Table 1). Birds were housed in 16 pens (1.3 m × 1.2 m) with wood shavings as bedding. The temperature was maintained at 33 ± 1 °C during the first week and reduced gradually to 24 ± 1 °C by the end of the trial. Relative humidity was maintained at 60–70% in week 1 and 50–60% thereafter. Lighting was provided using automated Ecko 400 W solar-LED floodlights. Birds were vaccinated against Newcastle disease (days 7 and 21) and Gumboro disease (days 14 and 28).

Growth performance: Birds were weighed weekly to establish body weight gain (final weight initial weight). Feed intake was calculated as feed offered minus feed refused. Feed conversion ratio (FCR) was computed as total feed intake divided by body weight gain.

Table 1. Ingredients and nutrient composition of the experimental basal diets

Ingredients (kg)	Starter (1–21 d)	Finisher (22–49 d)
Maize	50.50	56.00
Soybean	36.00	30.00
Maize gluten meal	6.00	6.00
Fish Meal	3.50	2.00
Soybean oil	1.50	3.55
Limestone	1.50	1.50
NaCl	0.25	0.25
Vitamin-mineral premix*	0.25	0.25
Lysine	0.20	0.15
Methionine	0.30	0.30
Total	100.00	100.00
Calculate composition (%)		
Crude protein	23.00	20.00
Crude fibre	4.68	3.94
Calcium	1.24	1.15
Available phosphorus	0.79	0.73
Lysine	1.27	1.08
Methionine	0.53	0.58
Metabolizable energy (MJ/kg)	12.25	13.18

*Vitamin-mineral premix (per 1kg) contains antioxidant (500 mg); biotin (300 mg); chlorine chloride (70000 mg); cobalt (80 mg); copper (1200 mg); folic acid (200 mg); iodine (400 mg); iron (8000 mg); manganese (16000 mg); niacin (8000 mg); pantothenic acid (2000 mg); selenium (80 mg); vitamin A (3400000 IU); vitamin B1 (640 mg); vitamin B12 (4 mg); vitamin B2 (16000 mg); vitamin B6 (600 mg); vitamin D3 (60000 IU); vitamin E (4000 mg); vitamin K3 (600 mg); zinc (12000 mg).

Carcass Evaluation: At day 49, two birds per replicate (n = 8 per treatment) were fasted for 12 h, slaughtered humanely, and eviscerated. Live weight, carcass weight, and carcass yield (%) were determined. breast weight, drumsticks, thighs, liver, heart, spleen, and kidneys were recorded Blood Sampling and analysis.

On day 49, three birds per replicate (n = 12 per treatment) were randomly selected for blood collection from the wing vein. Samples were collected into EDTA tubes for hematological analysis and plain tubes for serum biochemical and immune evaluation. Hematological indices (packed cell volume, red blood cell count, hemoglobin concentration, and white blood cell count) were determined using a LaserCyte® Hematology Analyzer. Serum biochemical indices (cholesterol, triglycerides, albumin, total protein, creatinine, urea, sodium, potassium, calcium, and magnesium) were analyzed using a VetTest® Chemistry Analyzer (IDEXX Laboratories). Thyroid hormones (triiodothyronine (T3), thyroxine (T4), and thyroid-stimulating hormone (TSH) were measured using a Biobase® ELISA plate reader.

Statistical Analysis: Data were analyzed using one-way analysis of variance (ANOVA) in SAS software (version 9.4; SAS Institute, Cary, NC, USA). Means were separated using Tukey's Honestly Significant Difference (HSD) test at $p < 0.05$. Linear and quadratic responses to increasing AP and BP inclusion were examined using orthogonal polynomial contrasts within the General Linear Model (GLM) procedure.

RESULTS AND DISCUSSION

Growth performance: There is no significant different in initial body weight among treatment groups, indicating uniform starting conditions (Table 2). Final body weight was significantly different in dietary different treatments ($p = 0.001$), with birds fed 1.5g/kg AP and 1.5g/kg BP (T4) achieving the highest values. This group also revealed the greatest total feed intake and body weight gain ($p = 0.001$). Daily feed intake per bird was highest in T4, suggesting enhanced appetite at higher supplementation levels. Feed conversion ratio did not differ significantly among treatments ($p = 0.158$), indicating that increased growth in T4 was primarily due to greater feed consumption rather than improved feed efficiency.

Carcass traits: Dietary supplementation significantly influenced dressed weight ($p = 0.030$), with T4 performing more than the control (T1) and moderate inclusion levels. No significant differences were observed for live weight, death weight, or defeathered weight ($p > 0.05$), However, increases were noted in T4. Among primal cuts, neck ($p = 0.048$), breast ($p = 0.036$), drumstick ($p = 0.019$), and thigh ($p = 0.020$) weights were significantly greater in T4, indicating improved muscle accretion. Internal organ weights were largely unaffected, except for a tendency toward higher liver weight in T3 compared to T1 ($p = 0.094$).

Hematological indices: Most hematological indices,

Table 2. The Effect of Alligator Pepper and Black Pepper on Growth Performance of Broilers

Parameters	Treatment 1	Treatment 2	Treatment 3	Treatment 4	SEM	P-value
Initial body weight (g)	351	351	351	351	0.18	1.000
Final body weight(g)	25531.50	25447.00	25765.50	28440.25[a]	375.91	0.001
Total feed intake (g)	60986.5	60131.25	62829.00[b]	65649.25[a]	643.58	0.001
Body weight gain (g)	25180.50	25096.00	25414.50	28089.25[a]	375.89	0.001
Average daily feed intake (g)	1089.04	1073.77	1121.95[b]	1172.31[a]	1089.77	0.001
Average daily feed intake/birds (g)	121.00	119.31	124.66[b]	130.26[a]	121.08	0.001
Feed conversion ration	2.43	2.39	2.47	2.34	2.36	0.158

note: a, b, c means with different superscripts along rows are significantly different ($p<0.05$)

including packed cell volume, red blood cell count, and total white blood cell count, were unaffected by treatments ($p > 0.05$) (Table 4). Hemoglobin concentration differed significantly ($p = 0.016$), with T1 and T2 has the highest values and T3 the lowest. Neutrophil percentage was highest, while eosinophil count were lowest in T4 compared to T1; other leukocyte types showed no significant variation.

Serum biochemistry: Dietary AP and BP inclusions significantly lowered serum triglyceride levels ($p < 0.05$), with all supplemented groups recording lower values than the control (Table 4). No significant changes were seen in total protein, albumin, cholesterol fractions, or alkaline phosphatase ($p > 0.05$). Creatinine levels was higher in T3 and T4 ($p = 0.092$), while urea levels showed a non-significant decrease in supplemented groups ($p = 0.107$). Uric acid remained unaffected ($p = 0.588$).

Immune response: Thyroxine (T4) concentration was significantly influenced by dietary treatment ($p = 0.037$), with T3 revealed the highest values. Triiodothyronine (T3) and thyroid-stimulating hormone (TSH) were unaffected ($p > 0.05$) (Table 5). These results suggest that moderate AP and BP inclusion may enhance thyroid activity without altering other endocrine markers.

The withdrawing of antibiotic growth promoters in poultry production, due to health concerns over antimicrobial resistance and consumer demand for residue-free products, has boost interest in phytogenic feed additives as viable alternatives (Abd El-Hack *et al*. 2022; Naderiboroojerdi *et al.* 2022; Alagawany *et al.* 2023). The present study examined the combined effects of AP and BP on broiler growth performance, carcass characteristics, hematological indices, and selected biochemical and immune indices.

Growth performance: The highest final body weight, weight gain, and feed intake were seen in birds fed 1.5g/kg each of AP and BP (T4), aligning with recent findings that phytogenic blends rich in alkaloids, flavonoids, and essential oils can improve digestive efficiency and nutrient absorption in poultry (Kilonzo-Nthenge *et al.* 2021; Al-Khalaifah, 2023). Piperine, the major bioactive in black pepper, has been reported to reviving digestive enzyme activity and enhance nutrient bioavailability (Kumar *et al.* 2022), which might be responsible for the improved growth in T4. The increase in feed intake could also be associated to the strong aroma and flavor of the spices, as supported by El-Sayed *et al.* (2021). However, feed conversion ratio did not differ significantly among treatments, contrary to some reports of improved FCR at lower inclusion rates of black pepper (0.5–1.0g/kg) (Rahman *et al.* 2021). Such differences may be dose-related or influenced by dietary composition and rearing conditions. This may also be indicative of that while the spices enhanced growth through possible stimulation of digestive secretions and nutrient utilization, the magnitude of improvement in feed efficiency was insufficient to reflect in FCR. Additionally, under standard commercial feeding conditions, where diets

Table 3. showing the effect of alligator pepper and black pepper on the hematology parameter of broilers

Parameters	T1	T2	T3	T4	SEM	P value
Packed cell volume (%)	28.50	28.50	29.00	27.80	0.49	0.78
White blood cell ($x10^9$/L	11.75	11.75	10.00	14.73	0.83	0.23
Red blood cell ($x12^{10}$/L)	318.00	317.50	324.50	331.25	5.71	0.84
Haemoglobin (g/dL)	8.50[a]	8.50[a]	7.00	7.75	0.21	0.016
Neutrophil (%)	61.00	49.50	57.00	66.50	3.15	0.29
Lymphocyte (%)	29.00	40.50	32.50	26.50	2.61	0.26
Monocyte (%)	6.50	8.00	8.00	5.50	0.52	0.25
Eosinophil (%)	3.50	1.50	2.00	1.00[c]	0.47	0.32
Basophil (%)	0.00	0.50	0.00	0.50	0.17	0.59

Mean values in the same row with different superscripts differ significantly ($p < 0.05$)

Table 4. The effect of alligator pepper and black pepper on the serum biochemistry

Parameters	T1	T2	T3	T4	SEM	P-value
Total Protein	50.75	50.75	56.75	55.25	1.04	0.062
Albumin	21.50	22.00	23.50	25.00	0.52	0.056
Total Cholesterol	3.25	4.25	3.50	3.25	0.18	0.161
Triglycerides	1.75[a]	1.00	1.00	1.00	0.10	0.002
HDL	2.00	2.00	2.50	2.25	0.10	0.248
LDL	1.00	1.25	0.25	0.75	0.16	0.159
Alkaline Phosphatase	267.75	270.75	275.50	252.50	4.37	0.290
Aspartate Aminotransferase	128.25	133.00	138.00	138.50	5.74	0.930
Alanine Aminotransferase	18.50	17.00	18.00	19.50	0.47	0.320
UREA	4.00	1.00	1.25	1.00	0.53	0.107
CREATINE	8.09[c]	13.54[b]	17.21[a]	17.17[a]	2.61	0.092
URIC ACID	0.25	0.00	0.25	0.00	0.09	0.588

Note: a, b, c means with different superscripts along rows are significantly different ($p<0.05$)

are already balanced, small enhancements in digestion or metabolism may not significantly affect feed efficiency (Abd El-Hack *et al.* 2023; Ijoma *et al.* 2024).

Carcass traits: T4 also yielded higher carcass traits, with better dressed weights and breast muscle yields, similar to the outcomes reported for phytogenic mixtures containing essential oils and alkaloids (Abd El-Hack *et al*. 2022; Kim *et al*. 2023). The combination of AP's anti-inflammatory flavonoids and BP's nutrient absorption enhancing piperine might have contributed to these effects. Absence of significant changes in organ weights, except for a slight liver weight increase in T3, supports the safety of the inclusion levels used, as reported in recent safety study of phytogenics in poultry (Al-Khalaifah, 2023).

The results also showed that AP and BP meal supplementation exerted notable effects on certain slaughter parameters.Birds receiving combined levels of alligator pepper and black pepper exhibited improved dressing percentage and carcass yield compared with the control. Mild increases in gizzard weight were also observed, potentially due to enhanced digestive stimulation by the AP and BP meal pungent phytochemicals. No adverse effects on liver, heart, or kidney weights were recorded, implying that the spices exerted no organ toxicity at the tested levels.

Hematology and serum biochemistry: Hematological indices remained within normal ranges, suggesting no deleterious effects on blood health, which was aligned with recent reports on phytogenic safety in broilers (Kumar *et al*. 2022; Alagawany *et al.* 2023). The lower eosinophil counts in T4 may be indicative of anti-inflammatory activity linked to the bioactive compounds present in both spices. Serum biochemistry revealed significantly reduced triglyceride levels across all supplemented groups, corroborating evidence that piperine can inhibit lipogenesis and improve lipid metabolism (Rahman *et al.* 2021; Kim *et al.* 2023). The stability of cholesterol fractions and protein parameters suggested that lipid modulation occurred without compromising protein metabolism or liver and kidney function. Slightly raised creatinine in T3 and T4 could be due to greater muscle deposition, consistent with the carcass yield findings.

The slight reduction in hemoglobin levels observed at the highest inclusion levels of alligator pepper and black pepper may indicate mild interference with erythropoiesis or iron utilization. Although phytochemicals such as tannins and alkaloids present in both spices can reduce iron absorption or alter red blood cell turnover, the recorded values in this study remained within the normal physiological range for broiler chickens, suggesting no clinically detrimental effect. The marginal increase in creatinine at maximum spice inclusion might be a reflection of increased muscle metabolism or the renal excretion of spice-derived metabolites. However, creatinine values also remained within accepted reference limits for healthy broilers, indicating that kidney function was not compromised.

Immune and endocrine responses: The rise in serum thyroxine in T3 aligns with reports that small phytogenic inclusion can stimulate thyroid activity and metabolism

Table 5. The effect of alligator pepper and black pepper on broiler immunological parameters

Parameters	T1	T2	T3	T4	SEM	P– value (<0.05)
Triiodothyronine (ng/ml)	2.05	2.75	2.70	2.70	0.125	0.129
Thyroxine (µg/ml)	0.53[b]	0.34[b]	0.65[a]	0.30	0.52	0.037
Thyroid-Stimulating Hormone (Iu/ml)	0.10	0.10	0.17	0.42	0.057	0.126

Note: A, b, c means with different superscripts along rows are significantly different ($p<0.05$)

(Abd El-Hack *et al.* 2022). However, the lack of a similar effect in T4 is suggestive of a dose-related feedback mechanism. No significant changes in triiodothyronine or TSH were observed, indicating a selective endocrine effect.

Mechanistic considerations and limitations: The improvements in growth and carcass yield may be due to a synergistic interplay between AP's anti-inflammatory and digestive-enhancing phytochemicals and BP's piperine mediated nutrient absorption. Reduced triglycerides and eosinophils further support lipid-lowering and anti-inflammatory effects. Even so, the absence of improvements in FCR suggests that further optimization of dosage and ratios is required. In addition, this study did not evaluate gut morphology, microbiota modulation, or oxidative stress status, which may identify the benefits of phytogenics in poultry (Al-Khalaifah, 2023; Kim *et al*. 2023).

While AP and BP are widely recognized for their antimicrobial and antioxidant properties, the present study did not assess gut microbiota shifts, pathogen reduction, or oxidative stress markers. Consequently, the health promoting effects discussed herein are based on literature backed biochemical properties rather than direct experimental evidence. This lack of microbiological and oxidative stress measurements is a limitation of the current work. Future research can incorporate microbial enumeration, intestinal morphometry, and antioxidant biomarkers such as malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and total antioxidant capacity to provide deeper mechanistic insight.

The dietary inclusion of 1.5g/kg each of alligator pepper and black pepper significantly improved growth and carcass traits without unfavorable impacts on hematology, biochemistry, or immune status. The findings support their potential as natural alternatives to antibiotic growth promoters. However, dose-response studies regarding gut health and oxidative stress markers under commercial production are recommended to improve their use in poultry nutrition.

DECLARATIONS

Ethics statement: This research was conducted with the approval of the Animal Welfare and Ethics Committee of the Department of Animal Production and Health, Federal University Oye-Ekiti (approval number: APH/R-010/20/11/26), and adhered to the ethical guidelines for the use of animals in research. This study involved no clinical trial; hence, clinical trial registration is not applicable.

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